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FORMULATION AND EVALUATION OF POLY-HERBAL ANTI-ACNE FACEWASH

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ABSTRACT: Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit that affects the major oil gland-containing areas, such as the face, back, and trunk. It involves increased sebum production and hair follicles abnormally desquamate as a result of rising androgen levels that coincide with puberty. It is generally characterized by, the formation of seborrhea, comedones, inflammatory papules, pustules, nodules, and lesions. *Propionibacterium acnes* and *Staphylococcus epidermidis* have been identified as bacteria that cause acne inflammation by generating pus. *Staphylococcus aureus* contributes to causing inflammation in acne. Herbal formulations have growing demand in the world market So, the current research focuses on developing and assessing a herbal face wash against the acne vulgaris and its etiologic agent. The ethanolic extract of Turmeric (rhizomes), Neem (leaves), Black pepper (fruit) and Nutmeg (fruit) was prepared by using a maceration process and formulated into a topical gel. The plants have been used in the formulation the having good anti-microbial, anti-oxidant and anti-inflammatory activity. The gel was formulated with different concentrations of the extract by dispersion method and the prepared formulation has been evaluated for a number of parameters like odor, color, appearance, consistency, pH, spreadability, wash ability, skin irritancy, and antimicrobial activity. The agar well diffusion method was used to test *in-vitro* antibacterial activity against *P. acnes*, *S. epidermidis*, and *S. aureus*. The produced formulation's-zones of inhibition were compared with a conventional antibiotic (Clindamycin) or herbal formulation.

INTRODUCTION: Acne vulgaris is the most common chronic inflammatory skin disorder of the pilosebaceous unit. It is a nearly universal condition that affects 95 % of males and 83 % of females of age 11-25 years ¹.

It mainly indicates non-inflammation causing open comedones (blackheads), closed comedones (whiteheads), and inflammation causing various conditions such as papules, pustules, and nodules ².

The micro-organisms responsible for acne vulgaris are, *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. These micro-organisms proliferate rapidly which ultimately results in the development of acne. Practically, symptoms of acne can be classified according to their intensity i.e. mild, moderate and severe ³.

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Propionibacterium acnes, an anaerobic bacteria, plays an important role in the pathogenesis of acne. It has the ability to metabolize sebum triglycerides into fatty acids, which chemotactically attract neutrophils and leads to the development of inflammatory acne⁴. In the sebaceous unit, *Staphylococcus epidermidis*, an aerobic bacteria that is part of the natural skin flora, is frequently involved in superficial infection⁵. *Staphylococcus aureus* is an anaerobic bacteria on the skin, nose, and other body surfaces and may cause, abscesses (boils), furuncles, and cellulitis⁶. The acne-causing bacteria such as *P. acnes*, *S. epidermidis* and *S. aureus* have become more resistant as a result of long-term overuse of antibiotics. Antibiotic resistance is caused by a variety of variables, including the nature of bacteria's, contact with antibiotics, how antibiotics are used, host features and environmental factors. Medical plants have been studied as alternative treatments for acne vulgaris in order to overcome the problem of antibiotic resistance.

Causes of Acne Vulgaris:

- Hyperactive sebaceous glands (overactive lipid secretion).
- Hyperkeratosis (accelerated keratinization) at hair infundibulum.
- The activity of bacteria (*Propionibacterium acnes*) promotes comedogenesis.
- Cyclic hormonal levels in women.
- Follicular plugging with sebum, keratinocytes, and inflammation.
- Occupational hazards include chronic exposure to chemicals, air contaminants, and high humidity.
- Hormones, environmental factors as well as genetic susceptibility may be the cause of acne.

Other events also associated with acne include, Seasonal effects, Excessive sexual activity, Emotional or Psychological stress and Mechanical manipulation of the skin surface⁷.

The Sequence of Events in Acne Vulgaris: Acne develops when hair follicles become clogged with dead skin cells and the sebaceous glands generate a sticky material called sebum. Inside the follicle due

to the excessive sebum secretion skin cell stick together and causes an obstruction. This leads to a comedone. When bacteria colonize a clogged pore or comedone, they release inflammatory factors. This causes comedones to turn into pimples and pustules. Some acne lesions swell up to the point of rupture, which forms nodules^{8,9}.

MATERIALS AND METHODS:

Plants Material:

Collection and Drying of Plant Parts: The dried rhizomes of Turmeric (*Curcuma longa*), Leaves of Neem (*Azadirachta indica*), fruits of Black pepper (*Piper nigrum*) and Nutmeg (*Myristica fragrans*) were collected from the local market of the district. The plant specimens were dried under shade. The leaves of neem, the fruit of black pepper, and nutmeg ground to get powder, and rhizomes of turmeric were cut into slender pieces, dried and then powdered. 70% Ethanol was required for the extraction of the plant specimens which, were collected from the college store^{10,11}.

Preparation of Plant Extract:

Cold Maceration: For the extraction we use, cold maceration. In that, the desired quantity of herbal drugs was weighed and each herb was macerated with ethanol (70%) in a conical flask. Allow phytoconstituent to mix with ethanol by moderate shaking of the conical flask for 3 days^{12,13}.

Filtration: After 3 days, the content was filtered out by using the simple filtration method. In that, filtration of the extract was done by using simple filter paper and a funnel.

Evaporation: Evaporation is done by using an electronic water bath. The above filtrate was allowed to evaporate in the pan at 60°C temperature until the desired quantity of extract was obtained **Fig. 1A and 1B**.



FIG. 1A: EXTRACT OF TURMERIC (CURCUMA LONGA), NEEM (AZADIRACHTA INDICA)



FIG. 1B: EXTRACT OF BLACK PEPPER (*PIPER NIGRUM*) AND NUTMEG (*MYRISTICA FRAGRANS*)

Evaluation of Extract:

Characteristics of Extract: The ethanolic extract of Turmeric, Neem, Black pepper, and Nutmeg was evaluated for its color, odor, taste, and physical state **Table 1**.

TABLE 1: CHARACTERISTICS OF EXTRACT

| Sr. no. | Characteristic | Turmeric | Neem | Black pepper | Nutmeg |
|---------|----------------|-----------|----------------|----------------|----------------|
| 1 | Physical state | Semisolid | Semisolid | Semisolid | Semisolid |
| 2 | Color | Yellowish | Greenish | Blackish | Brownish |
| 3 | Odor | Aromatic | Aromatic | Aromatic | Aromatic |
| 4 | Taste | Bitter | Characteristic | Characteristic | Characteristic |

TABLE 2: PHYTOCHEMICAL SCREENING OF THE EXTRACT

| Sr. no. | Phytoconstituent | Turmeric | Neem | Blackpepper | Nutmeg |
|---------|------------------|----------|------|-------------|--------|
| 1 | Phenol | + | + | + | + |
| 2 | Tannins | + | + | + | + |
| 3 | Steroids | - | + | + | - |
| 4 | Alkaloids | + | + | + | + |
| 5 | Glycosides | + | + | + | - |
| 6 | Flavonoids | + | + | + | + |

TABLE 3: EVALUATION OF ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT

| Sr. no. | Bacteria | Zone of Inhibition (mm) | | | | Combination | Standard Clindamycin |
|---------|-------------------------------|-------------------------|----------|--------------|--------|-------------|----------------------|
| | | Neem | Turmeric | Black pepper | Nutmeg | | |
| 1. | <i>Propionibacterium acne</i> | 12 | 9 | 10 | 7 | 17 | 36 |

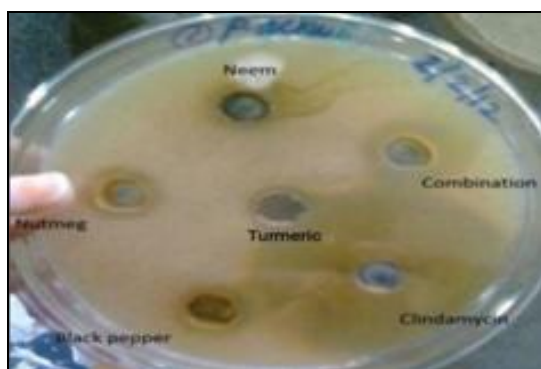


FIG. 2: EVALUATION OF ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT

METHODS:

Procedure: Dissolve 1 gm of carbopol 940 in 50 ml of distilled water with continuous stirring. Kept the beaker aside to swell the carbopol 940 to form a

Phytochemical Screening of Extract: The ethanolic extract of Turmeric, Neem, Blackpepper, and Nutmeg were subjected to preliminary phytochemical testing for the detection of major phytoconstituent such as phenols, tannins, alkaloids, glycoside, flavonoids, etc **Table 2**.

Antimicrobial Activity of Plant Extract: The ethanolic extracts of turmeric, neem, black pepper, and nutmeg and their combination were examined for antimicrobial activities against microorganisms frequently involved in acne inflammation, i.e. *Propionibacterium acnes* respectively. It was observed that the combination of all four extracts shows the maximum zone of inhibition as compared to individual extracts ¹⁴ **Table 3, Fig. 2**.

gel. Take 5 ml of distilled water and heat it in a water bath to dissolve the necessary amount of methylparaben and propylparaben. Allow the solution to cool and then, add the required quantity of propylene glycol 400, Polyethylene glycol 200, and sodium lauryl sulfate.

Further required quantity of ethanolic extract of Turmeric, Neem, Black pepper, and Nutmeg was mixed into the above mixture and the volume was made up to 50 ml by adding the remaining distilled water. Finally, after mixing all ingredients properly to the carpool 940 gel with continuous stirring add triethanolamine dropwise to the formulation for the adjustment of the required skin pH (6.8 - 7) and to obtain the gel of the required consistency.

The same method was followed for another formulation having different concentrations of plant extract¹⁵⁻¹⁸ **Fig. 3.**

Evaluation of Facewash:

Physical Evaluation: Physical parameters such as physical state, color, odor, taste and appearance we rechecked visually and results are displayed in **Table 6.**

Measurement of pH: The pH of various formulations was determined by using a Digital pH meter. One gram of gel was dissolved in 100 ml of distilled water and stored for two hours. The measurement of the pH of each formulation was done in triplicate and calculated the average¹⁹. The average values of each formulation are displayed in **Table 7, Fig. 4.**

TABLE 4: FORMULATION OF GEL

| Sr. no. | Ingredient Name | Quantity (50gm) | |
|---------|-------------------------|-----------------|-----------|
| | | F1 | F2 |
| 1 | Carbopol 940 | 1 | 1 |
| 2 | Methylparaben | 0.150 | 0.150 |
| 3 | Propylparaben | 0.030 | 0.030 |
| 4 | Polyethylene glycol 400 | 15 | 15 |
| 5 | Propylene glycol | 5 | 5 |
| 6 | Sodium Lauryl Sulphate | 2.5 | 2.5 |
| 7 | Distilled water | Upto50 ml | Upto50 ml |

TABLE 5: THE CONCENTRATION OF EXTRACT USED IN GEL

| Sr. no. | Plant Extract | Quantity (50 gm gel) | |
|---------|---------------------|----------------------|------|
| | | F1 | F2 |
| 1 | Turmeric Extract | 0.25 | 0.25 |
| 2 | Neem Extract | 0.75 | 1.50 |
| 3 | Blackpepper Extract | 0.87 | 1.75 |
| 4 | Nutmeg Extract | 0.25 | 0.5 |



FIG. 3: FORMULATIONS OF POLYHERBAL ANTI-ACNE FACEWASH

TABLE 6: PHYSICAL EVALUATION

| Sr. no. | Parameters | Observation |
|---------|----------------|-----------------|
| 1 | Physical state | Semisolid |
| 2 | Colour | Yellowish brown |
| 3 | Odour | Characteristic |
| 4 | Taste | Characteristic |
| 5 | Smoothness | Smooth |



FIG. 4: PH OF FORMULATION ON PH PAPER AND DIGITAL PH METER

Viscosity: The measurement of the viscosity of prepared gels was carried out with Brookfield Viscometer the measurements were over a speed setting of 100 rpm at 25 °C using Brookfield Viscometer. The values of each formulation are displayed in **Table 7**.

Spreadability: It denotes the extent of the area to which the gel readily spreads on application to the skin or the affected part. The bioavailability efficiency of a gel also depends on its spreading value. The spreadability was expressed in terms of time in seconds taken by two slides to slip off from the gel, placed in between the slides, under a certain load. Lesser time taken for a slip of the two slides means that it has better spreadability^{1,2}. The values of each formulation are displayed in **Table 7**. Glass slides with uniform dimensions were selected from two sets. One of the slides was covered with the gel formulation. The gel was sandwiched between the two slides in a region that took up a distance of 6.0 cm along the slide when the other slide was positioned on top of the gel. The upper slides were given a 100 gm weight so that the gel between the two slides would be uniformly compressed to produce a thin layer. The excess gel that was sticking to the slides was scraped off after the weight was taken off. The two slides were securely fastened to a stand so that there was no least movement and that only the upper slide could be released by the weight that was fastened to it. A 20 g weight was securely fastened to the upper slide. Under the impact of the weight, it took 6.0 cm of time for the higher slide to go upward and separate from the lower slide. The experiment was done three times, and the average computation time was recorded. The following formula was used to determine spreadability,

$$S = M L / T$$

Where, S is for spreadability, M is for weight in the pan (attached to the upper slide), L is for glass slide length, and T is for the amount of time (in seconds) needed to separate the slides.

Washability: Formulations were applied on the skin and then the ease and extent of washing with water were checked manually. The formulation is easily washed with water which means that it is easily washable.

Irritancy Test: The cream was applied on the left-hand dorsal side surface of 1 sq. cm and observed in equal intervals up to 24 hrs for irritancy, redness, and edema. There is no signs of irritancy, redness, and edema have been observed.

Antimicrobial Activity: In this antimicrobial activity, formulations were checked against the microorganisms frequently involved in acne inflammation, i.e., *Propionibacterium acnes*. The modified agar well diffusion method determined the formulations' antibacterial activity¹⁴ **Table 8, Fig. 5.**

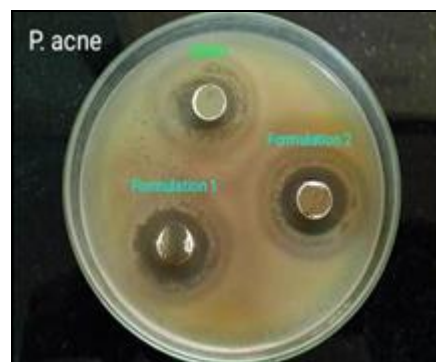


FIG. 5: ANTIMICROBIAL ACTIVITY OF VARIOUS FORMULATIONS AGAINST P. ACNES

TABLE 7: EVALUATION OF FACEWASH

| Sr. no. | Formulations | Appearance | pH | Viscosity (cps) | Spreadability (gm-cm/sec) |
|---------|--------------|-----------------|------|-----------------|---------------------------|
| 1 | F1 | Yellowish brown | 6.19 | 3640 | 10.34 |
| 2 | F2 | Yellowish brown | 6.39 | 3690 | 11.45 |

TABLE 8: ANTIMICROBIAL ACTIVITY OF VARIOUS FORMULATIONS AGAINST P. ACNES

| Sr. no. | Formulations | Zone of inhibition (mm) |
|---------|--------------|-------------------------|
| 1. | F1 | 12 |
| 2. | F2 | 17 |

Comparative Study: The plates were dried for 1 hr. With sterile 8 mm borer wells in each of the plates were cut into which 0.5 ml of solutions of

prepared polyherbal facewash, marketed herbal formulation, and standard clindamycin were introduced in the plate. The antibacterial activity

was evaluated by measuring the diameter of zones of inhibition (in mm)²⁰. The experiments were repeated two times and the mean was calculated **Table 9, Fig. 6.**



FIG. 6: ANTIMICROBIAL ACTIVITY OF VARIOUS FORMULATIONS AGAINST P. ACNES

TABLE 9: ANTIMICROBIAL ACTIVITY OF VARIOUS FORMULATIONS AGAINST P. ACNES

| Sr. no. | Different formulations | Zone of inhibition (mm) |
|---------|-------------------------------|-------------------------|
| 1 | Prepared polyherbal face wash | 17 |
| 2 | Marketed herbal formulation | 19 |
| 3 | Clindamycin phosphate | 36 |

RESULT: The present research work deals with formulating and evaluating herbal anti-acne facewash. The plant material used for the formulations was ethanolic extract of, rhizomes of *Curcuma longa* (turmeric), leaves of *Azadirachta indica* (neem), fruits of *Myristica fragrans* (nutmeg) and *Piper nigrum* (black pepper). Turmeric, Neem, Black pepper, and Nutmeg have been good antibacterial, antifungal, antioxidant, and anti-inflammatory agents. The developed formulations F1 and F2 were evaluated and the results of the evaluation are displayed in **Table 11.**

TABLE 11: RESULT OF EVALUATION OF POLYHERBAL ANTI-ACNE FACE WASH

| Sr. no. | Parameters | Observation | |
|---------|------------------------|-----------------|-----------------|
| | | F1 | F2 |
| 1 | Consistency | Semisolid | Semisolid |
| 2 | Viscosity | 3640 | 3690 |
| 3 | pH | 6.39 | 6.39 |
| 4 | Spreadability | 10.34 | 11.45 |
| 5 | Washability | Easily washable | Easily washable |
| 6 | Skin irritancy | Nosigns | Nosigns |
| 7 | Antimicrobial activity | Present | Present |

CONCLUSION: Natural remedies are more acceptable and they are safer with minimum side effects than synthetic ones. Herbal formulations have growing demand in the world market so, we trying to make a herbal formulation instead of a

The developed formulation are also evaluated for their in vitro antibacterial activity against *P. acnes*. The zones of inhibitions of the antibacterial activity were compared with the standard Clindamycin, marketed herbal anti-acne preparation.

TABLE 10: PHYSICAL EVALUATION

| Sr. no. | Parameters | Observation |
|---------|----------------|-----------------|
| 1 | Physical state | Semisolid |
| 2 | Colour | Yellowish brown |
| 3 | Odour | Characteristic |
| 4 | Taste | Characteristic |
| 5 | Smoothness | Smooth |

DISCUSSION: The present research work deals with formulating and evaluating herbal anti-acne face wash. The plant material used for the formulations was ethanolic extract of Turmeric, Neem, Black pepper and Nutmeg.

Although various topical herbal formulations for acne are available in the market, we propose to make use of turmeric first time with neem, black pepper, and nutmeg. Turmeric, neem, black pepper, and nutmeg have been reported in the literature as good antibacterial and anti-inflammatory agents.

The *in-vitro* antibacterial activity of the developed formulations against *P. acnes* was evaluated. The antibacterial zones of inhibition were compared to commercially available herbal anti-acne treatments like Clindamycin. The antibacterial efficacy of ethanolic extracts of neem, nutmeg, and black pepper against *P. acnes* was demonstrated but, the combination of all three extracts showed more zone of inhibition as compared to individual extracts.

synthetic one. The present research work deals with formulating and evaluating herbal anti-acne facewash. The plant material used for the formulations were ethanolic extract of, rhizomes of *Curcuma longa* (turmeric), leaves of *Azadirachta*

indica (neem), fruits of *Myristica fragrance* (nutmeg) and *Piper nigrum* (black pepper). It has good antimicrobial, antifungal, anti-inflammatory, and antioxidant activity. The formulations F1 and F2 are prepared by using different concentrations of extract and evaluated for their physicochemical properties like, color, odor appearance, spreadability, washability, irritation test, drug content, and antimicrobial activity. The research concluded that the developed herbal formulation of batch F2 comparatively showed better results than the other formulation.

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CONFLICTS OF INTEREST: Nil

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